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CO-PURIFICATION OF THE AMINOACYL-tRNA SYNTHETASE COMPLEX WITH THE ELONGATION FACTOR eEF1

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A multi-enzyme complex of mammalian aminoacyl-tRNA synthetases was isolated from rabbit reticulocytes, and purified by polyethylene glycol fractionation and gel filtration on Biogel A15m and affinity chromatography on tRNA-Sepharose. The synthetase complex contains nine synthetase activities, and the corresponding proteins as analyzed by SDS polyacrylamide gel electrophoresis. Three of the proteins showed the identical subunit molecular weights to those of the reticulocyte's elongation factor eEF1H. The eEF1α protein could not be removed by second tRNA-Sepharose column chromatography, or gel filtration on Biogel A5m or Biogel A15m. Antibodies against eEF1α react with the purified synthetase complex on the basis of dot blot analysis. This finding should provide new clues for elucidating the structural organization of the mammalian protein biosynthetic machinery.

At least eight of the mammalian aminoacyl-tRNA synthetases are associated as a multi-enzyme complex (for reviews, see 1, 2, 3). The functional significance of the synthetase complex has been a long standing question, despite intensive studies by a number of laboratories. The complex formation is not required for the enzymatic activities of the synthetases. The synthetases specified in the synthetase complex do not correlate with the genetic codons of the cognate amino acids, the amino acid transport systems or the mammalian amino acid nutritional requirements.

In this communication, we report the isolation of the synthetase complex with bound elongation factor. This finding should provide new clues for elucidating the function of the synthetase complex.

Materials and Methods

Un-fractionated calf liver and yeast tRNA's were from Boehringer-Mannheim. Radioactive [C-14] amino acids were purchased from ICN. Rabbit reticulocytes were purchased from Green Hectares

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(Wisconsin). Aminoacyl-tRNA synthetases were assayed by the initial rate of aminoacylation. Standard assay mixture contains in 50 µl, 200 mM Tris HCl (pH 7.5), 10 mM $MgCl_2$, 2 mM DTT, 50 µM [C-14] amino acid (150 µCi/µmole), 4 mM ATP, 0.2 µM EDTA, 0.2 mg/ml bovine serum albumin and a saturating amount of yeast or calf liver tRNA. Calf liver tRNA was used primarily for arginyl-, and aspartyl-tRNA synthetases. Acid precipitable radioactivity was determined as previously described (4). One unit is defined as 1 nmol of aminoacyl-tRNA formed per min. Elongation factors from rabbit reticulocytes and eEF1H polyclonal antibodies were generous gifts from Dr. William Merrick (Case Western Reserve).

The synthetase complex was purified from rabbit reticulocytes at 0-4°C. The reticulocytes were pelleted by centrifugation at 3,000 rpm for 10 min. The pellet was washed twice with phosphate buffered saline (0.15 M NaCl, 10 mM sodium phosphate, pH 7.5). The pelleted cells were resuspended in 2.5 volumes of freshly prepared lysis buffer (5 mM Tris-HCl (pH 7.5), 1 mM PMSF, 1 mM EDTA, 0.1 mM TLCK, 0.1 mM TPCK, 1 µM leupeptin, 1 µM pepstatin-A, 1 mM DTT, 10% glycerol), and centrifuged for 90 minutes at 37,000 rpm in a Beckman Ti40 rotor. The high speed supernatant was then precipitated by adding 50% polyethylene glycol 6000 (Sigma) to a final concentration of 8%. The resulting precipitate was redissolved in a minimum volume of buffer T (50 mM Tris HCl, pH 7.5, 2 mM DTE, 3 mM $MgCl_2$, 10% glycerol)containing all six protease inhibitors, and chromatographed on a column (63.5 x 2.5 cm) of Biorad Biogel A15m. Fractions of 7 ml were collected and assayed for synthetase activities. The fractions containing the high molecular weigh form of the synthetases were pooled and chromatographed on a column (1 x 6 cm) of E. coli tRNA-Sepharose. The tRNA-Sepharose column was successively washed with 3 column-volume each of buffer T and 50 mM KCl in buffer T, and the synthetases were eluted by six column-volumes of a linear gradient (0.05 to 0.6 M) of KCl. Fractions of 1 ml were collected and assayed for synthetase activity, and analyzed by SDS polyacrylamide gel electrophoresis. Active fractions were stored in liquid nitrogen.

Results

Reticulocytes were used for the purification of the synthetase complex, because of its relatively low levels of proteases as compared to other tissues such as liver. A battery of six protease inhibitors were included in buffers in all steps before gel filtration, instead of single unstable inhibitor such as PMSF or DIFP, which was used in the initial steps in previously reported purification procedures (4,5). These inhibitors include EDTA, TPCK, TLCK, leupeptin, pepstatin and PMSF. Gel filtration on Biogel A15m, which has an exclusion limit of 15 million, was used in order to resolve the dissociated synthetase complexes. As shown in Figure 1, two distinct activity peaks were partially resolved by gel filtration. Both peaks are resolved from the bulk of proteins fractionated by polyethylene glycol. The first activity peak, containing the large synthetase complex, was pooled and

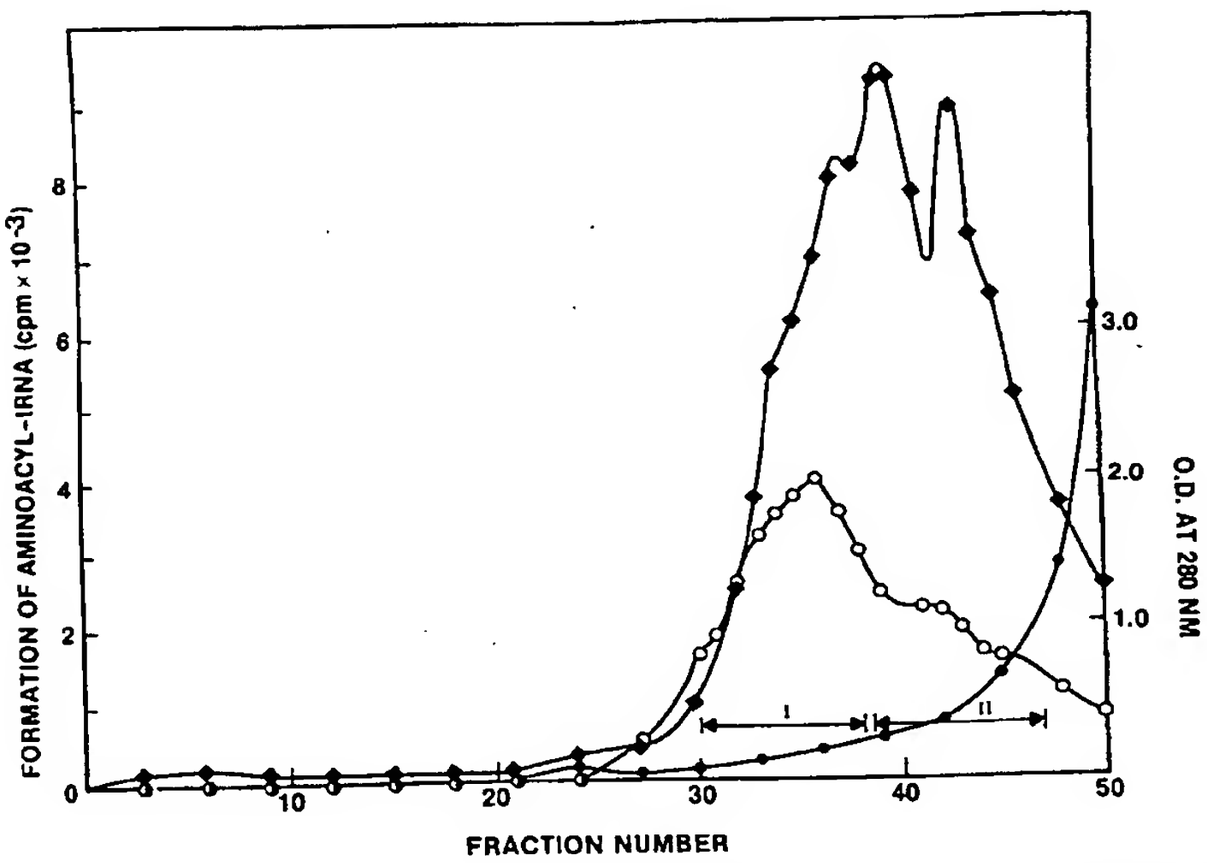

Figure 1. Gel filtration of polyethylene glycol fractionated reticulocyte extract on a column of Biogel A15m. Activities of lysyl- (◆) and valyl- (○) tRNA synthetases and absorbance at 280 nm (●) are analyzed as described in Material and Methods.

affinity purified on a column of E. coli tRNA-Sepharose. The fractions were assayed for synthetase activities and analyzed by SDS polyacrylamide gel electrophoresis (Fig. 2). Eight of the top

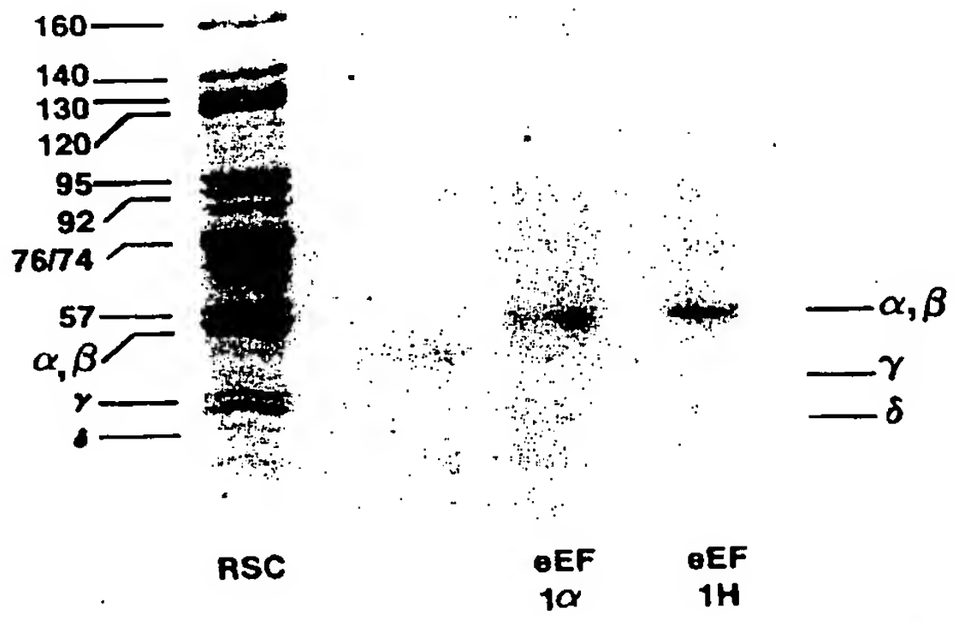

Figure 2. SDS polyacrylamide gel electrophoresis of the synthetase complex (RSC), eEF1α and eEF1H. The numbers on the left of the RSC lane correspond to the subunit molecular weights of the synthetase in kilodalton. The subunits of eEF1H are labeled as α, β, γ and δ.

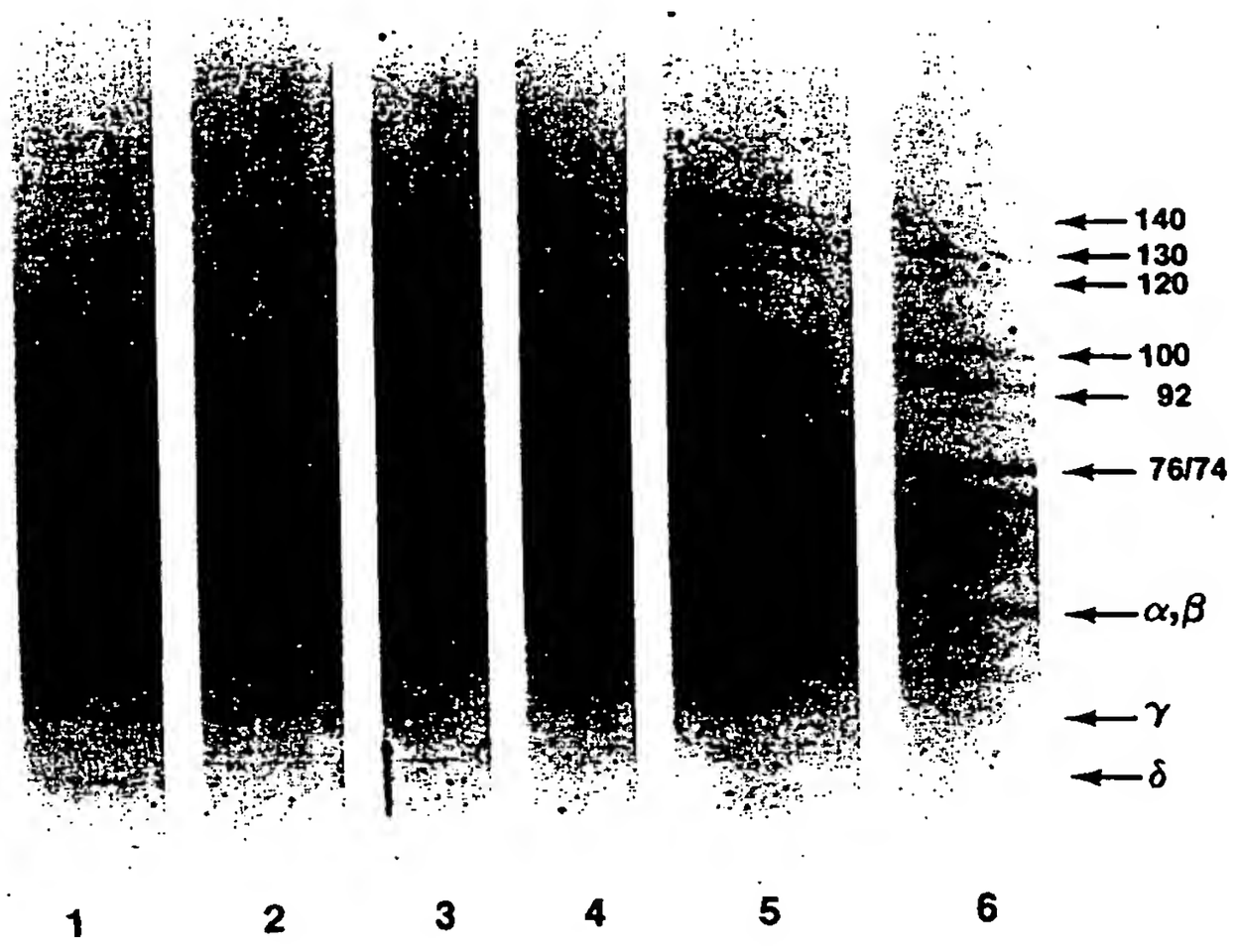

Figure 3. SDS polyacrylamide gel electrophoresis of the synthetase complex after a second column chromatography on tRNA-Sepharose. The salt (50 mM) wash is shown in lanes 1 and 2. The alternative fractions containing the synthetase complex are shown in lanes 3, 4, 5 and 6. The numbers on the right are the subunit molecular weights in kilodalton of the synthetases. The subunits of eEF1H are labeled as α, β, γ and δ.

nine proteins in this synthetase complex are comparable to the synthetase complex previously purified from a number of tissues and cells (4,5). Three low M_r proteins are proteins which were absent in the previously reported synthetase complex, and have molecular weights identical to the authentic elongation factor eEF1H (Fig. 2). These proteins co-purified through the steps of purification. Since eEF1α is a major cellular protein, the purified complex was re-chromatographed on a second tRNA-Sepharose affinity column to remove excess free eEF1α. Dissociated synthetases and elongation factor were eluted off the tRNA-Sepharose column by 50 mM KCl, while the core complex with the elongation factor was eluted at 0.25 M KCl by the salt gradient (Fig. 3).

Dot blot assay of the purified complex using polyclonal antibodies against eEF1α confirmed the presence of eEF1α. Since the polyclonal antibodies did not stain eEF1α proteins on the nitrocellulose blot, the presence of eEF1α could not be confirmed by western blot analysis at present.

The molecular weight of the purified synthetase complex is over 1 million when analyzed by gel filtration on Biorad Biogel A5m or A15m. The sedimentation coefficient of the purified complex is 22 S, as determined by sucrose gradient sedimentation. The molecular weight and the sedimentation coefficient are significantly greater than the previous purified 18 S synthetase complex (4,6). The purified complex has specific activities for arginyl-, glutaminyl-, isoleucyl-and lysyl-tRNA synthetases of 106, 49, 144 and 74 units/mg, respectively. These specific activities are higher than those of the previously purified synthetase complex (4). The various specific activities are of the same order of magnitude rather than a spread of two orders of magnitude.

Discussion

The co-purification of the elongation factor with the synthetase complex suggests their physical association. The successful demonstration is likely due to the presence of a battery of protease inhibitors during the purification. Synthetases are highly susceptible to endogenous proteases, as previously shown in the cases of methionyl- (7) and valyl-tRNA synthetases (8). A few fully active synthetases can be dissociated from the synthetase complex by controlled proteolysis. It's conceivable that cleavage of a small fragment in one of the synthetases in the complex can result in the dissociation of the elongation factor.

Since eEF1α is a major cellular protein (9), the possibility of contamination of the synthetase complex preparation by eEF1α was examined. Two observations suggest that eEF1α is indeed associated with the synthetase complex. First, the intensity of the eEF1H polypeptides is comparable to those of the synthetases in the SDS electropherograms of the purified complex, despite the fact that eEF1α is a major cellular protein. Secondly, synthetase complex binds tRNA-Sepharose more tightly than the elongation factor does, such that a small fraction of free eEF1 co-purified with the synthetase complex can be easily resolved from the synthetase complex. The synthetase complex co-elutes with the elongation factor after the second column chromatography on tRNA-Sepharose.

Co-purification of the synthetase complex with the elongation factor eEF1α suggests their physical association and possibly facilitates the transfer of aminoacyl-tRNA's from the synthetase complex to the elongation factor (Escalante and Yang, unpublished results). The mechanism of the re-cycling of eEF1α in the

synthetase complex during protein biosynthesis is yet to be examined. Channeling through compartmentalization of aminoacyl-tRNA's during protein biosynthesis has been suggested by the preferential incorporation of exogenous amino acids into nascent polypeptides (10) and poor incorporation of exogenous aminoacyl-tRNA (11).

Subcellular localization of the mammalian synthetases by direct and indirect immunofluorescence demonstrated the association of the majority of the synthetase complex with the detergent resistent cell matrix (12). One of the actin bundling protein ABP-50 has recently been identified as EF1α (13). The present demonstration of the co-purification of the synthetase complex with the EF1α suggests that EF1α may act as the bridging protein between the cytoskeleton and the synthetase complex, and provide the framework for the structural organization of the protein biosynthetic machinery in mammalian cells (14).

In summary, the association of the synthetases as a multi-enzyme complex appears to organize the synthetases such that aminoacyl-tRNA's as the products of synthetases may be collectively channeled to the elongation factor eEF1α. Direct evidence for such a scheme is yet to be established. The present result opens such a possibility and reveals one possible aspect of the structural basis of such a scheme in protein biosynthesis.

Acknowledgment

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